# Effects of clenbuterol on body stores of polychlorinated dibenzofurans (PCDF) and dibenzo-p-dioxins (PCDD) in rats

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# Effects of clenbuterol on body stores of polychlorinated dibenzofurans (PCDF) and dibenzo-*p*-dioxins (PCDD) in rats[1,2]

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**ABSTRACT:** Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), persistent pollutants that accumulate in the food chain, pose a risk to humans through consumption of tainted livestock. Clenbuterol, a leanness-enhancing agent, was tested for usefulness in PCDD/F body store reduction through body fat reduction (the predominant site of accumulation). To mimic the situation of contaminated animals, rats were given feed with or without a mixture of PCDD/F (0.6 to 2.7 ng/congener per day) for 10 d, followed by 16 d of feed with or without dietary clenbuterol (2 mg/kg feed). Clenbuterol reduced body fat by 28% ($P < 0.05$), increased muscle mass by 25% ($P < 0.02$), and decreased liver mass by 7% ($P < 0.02$). Although the concentrations of most PCDD/F per gram of fat were slightly increased after clenbuterol treatment, the total amount of PCDD/F that remained in fat was reduced by approximately 30%. Muscle PCDD/F concentrations and total burden were decreased by clenbuterol. In contrast, clenbuterol tended to increase concentration, but not total burden of PCDD/F in livers. One congener known to be rapidly metabolized and excreted, 2,3,7,8-TCDF, was the exception to this increase, decreasing 40% with clenbuterol treatment. This was also the congener that showed the greatest reduction in both fat and muscle. Examination of the ratio of PCDD/F in liver and fat revealed that clenbuterol increased the liver's share of the body burden of PCDD/F, from 38 to 75%. In a remediation/disposal context, these findings would be beneficial if clenbuterol lowered the meat and carcass burden of PCDD/F to safe levels, requiring only livers to be disposed of as hazardous waste.

Key Words: Clenbuterol, Dioxins, Furans, Rats

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## Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/F) have been found as contaminants in beef and chicken in the United States. Contact (rubbing or chewing) with pentachlorophenol-treated lumber has been implicated as one source of dioxin contamination in beef (Feil et al., 1997), while contaminated ball clay, used as an anti-caking agent in feeds, was confirmed as a source of poultry contamination (Hayward et al., 1999). In Belgium, millions of chickens were destroyed in 1999 after eating feed contaminated with dioxins and polychlorinated biphenyls, resulting in an estimated cost of $900 million (chemical analyses, lost revenues, and removal of contaminated products; the sequence of events was described in Bernard et al., 2002).

Few methods have been explored for the remediation of animals exposed to dioxins. Morita and coworkers (1993, 1997, and 1999) have looked at the effect of diet (including various green plants, fiber, seaweed, and a chlorophyll extract) on absorption and fecal excretion of PCDD and PCDF. This study reports the efficacy of using the leanness-enhancing agent, clenbuterol, in the remediation of dioxin-contaminated animals. Clenbuterol decreases body fat and increases muscle mass (sheep, Claeys et al., 1989; cattle, Schiavetta et al., 1990). Because the predominant site of dioxin and furan accumulation is fatty tissues, it was hypothesized that clenbuterol might lower body burdens with elimination of body fat. A remediation situation was mimicked by

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[4]Retired.

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feeding rats low levels of PCDD and PCDF, followed by treatment with clenbuterol. The resultant effect of clenbuterol on PCDD and PCDF stores in fat, muscle, and liver were examined. If a feeding regimen using clenbuterol was successful at lowering dioxins in feed animals, millions of dollars could be saved and a tainted food supply salvaged.

## Methods

### *Experimental Design*

Male Sprague-Dawley rats (n = 32, ~ 150 g, Harlan Sprague Dawley, Madison, WI) were individually housed, and weight gain and feed intake data over a 1-wk period were used to group animals in replicates (four groups, eight animals each). Within replicates, treatments were randomly assigned (individual housing was maintained at all times). For 10 d, 16 rats received ground feed fortified with corn oil (100 μL on 3 g of ground rat chow) that contained the PCDD/F congener mixture (dose and toxic equivalency factors [**TEF**] are listed in Table 1), and 16 rats were given feed fortified with pure corn oil. After fortified chow was consumed, ad libitum access to untreated feed was provided for a 7-h period. On d 11, rats were subgrouped (eight animals) and provided ad libitum access to rat chow with, or without, 2 mg clenbuterol/kg feed for 16 d. The corn oil supplementation was continued during this period because a preliminary experiment indicated that feed intake changed when oil was removed from the diet. Daily feed intake and body weights were recorded throughout. On d 27 rats were killed with carbon dioxide. A schematic of the experimental design is provided in Figure 1. Control animals were included to determine the endogenous body burden of PCDD/F (which are ubiquitous in the environment) at the beginning of the experimental period and to follow any changes in the concentration of these congeners with or without clenbuterol. At necropsy, tissue and organ weights were recorded and fat (epididymal, renal, and abdominal) dissected for later congener analysis. For feed intake, the body, organ, and tissue weight sample size was eight, whereas for PCDD/F analyses, it was four (one from each replicate). Whole muscles collected included semitendinosus, gluteus superficialis and medius, semimembranosus, biceps femoris, longissimus dorsi, and triceps brachialis. The animal protocol was approved by the Institutional Animal Care and Use Committee of the USDA-ARS Biosciences Research Lab. Safety precautions were followed as described in EPA Method 1613 (1990) for handling dioxin and furan samples and waste.

### *Tissue Processing and PCDD/PCDDF Analyses*

Tissues were processed by a modification of EPA Method 1613 as diagrammed (Figure 2). Liver from rats receiving no exogenous PCDD/F and all fat samples were analyzed at our location, while muscle and livers from PCDD/F-dosed animals were analyzed elsewhere. This was done to expedite sample analysis. Livers from control and clenbuterol-treated rats were extracted using an Accelerated Solvent Extractor (ASE Dionex, Sunnyvale, CA) in place of Soxhlet for extraction, giving results equivalent to the Soxhlet method (congener concentrations were an average of ±10% of the concentrations determined by Soxhlet). All samples were concentrated on a Turbo Vap II Concentration Work Station (Hymark Corp., Hopkinton, MA). Liquid-liquid extractions were performed until the aqueous phase was colorless. Rinse steps are not included in the figure, but were performed after container transfers. Samples processed on the FMS (Dioxin-Prep System, Fluid Management Systems, Waltham, MA) were fortified with [$^{37}$Cl]-2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) prior to loading to assess FMS recovery. Silica and alumina FMS columns were conditioned with hexane. Carbon columns were conditioned with the following solvent series: toluene, ethyl acetate:benzene (1:1), methylene chloride:hexane (1:1), and hexane. After sample application, the silica column was washed with hexane which flowed onto the alumina column, followed by a 2% methylene chloride elution. The PCDD/F were eluted onto the carbon column using methylene chloride:hexane (1:1). The carbon column was flushed with ethyl acetate:benzene (1:1), followed by hexane. Direction of flow was reversed and PCDD/F were eluted from the top of the column with toluene. Internal [$^{13}$C]-standards ([$^{13}$C]-1,2,3,4-TCDD and [$^{13}$C]-1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin [H×CDD]) were added to samples before high resolution (HR) GC-MS analyses according to EPA Method 1613 (EPA, 1990). All PCDD/F standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada). Celite was obtained from Fisher Scientific (Pittsburgh, PA). Matrix blanks with and without a standard mixture of native PCDD/F were included for all FMS sets to assess the quality of the analytical method. Muscle from rats not receiving PCDD/F were not analyzed because values would have been below the limits of detection. Due to expense (~$800 per sample), congener analysis was done on tissues from one animal per treatment replicate (n = 4 per treatment). Seventeen congeners of PCDD/F are identified by the (HR) GC-MS analysis. Congeners that were given to rats in the corn oil are denoted "dosed congeners" (nine congeners), and those congeners not present in the dose (reflecting environmental ubiquitous exposure) are denoted "non-dosed congeners" (Table 1). Of the eight non-dosed congeners analyzed, all were < 0.05 ng in total dose with the exception of 2,3,4,6,7,8 H×CDF (0.15 ng) and 1,2,3,7,8,9 H×CDD (0.38 ng). Numerical identification is used only for non-dosed congeners (there was only one congener per chlorination number in the dosed set).

### *Statistical Analysis*

Clenbuterol effect on congener data was analyzed using a mixed model analysis of variance (SAS/STAT,

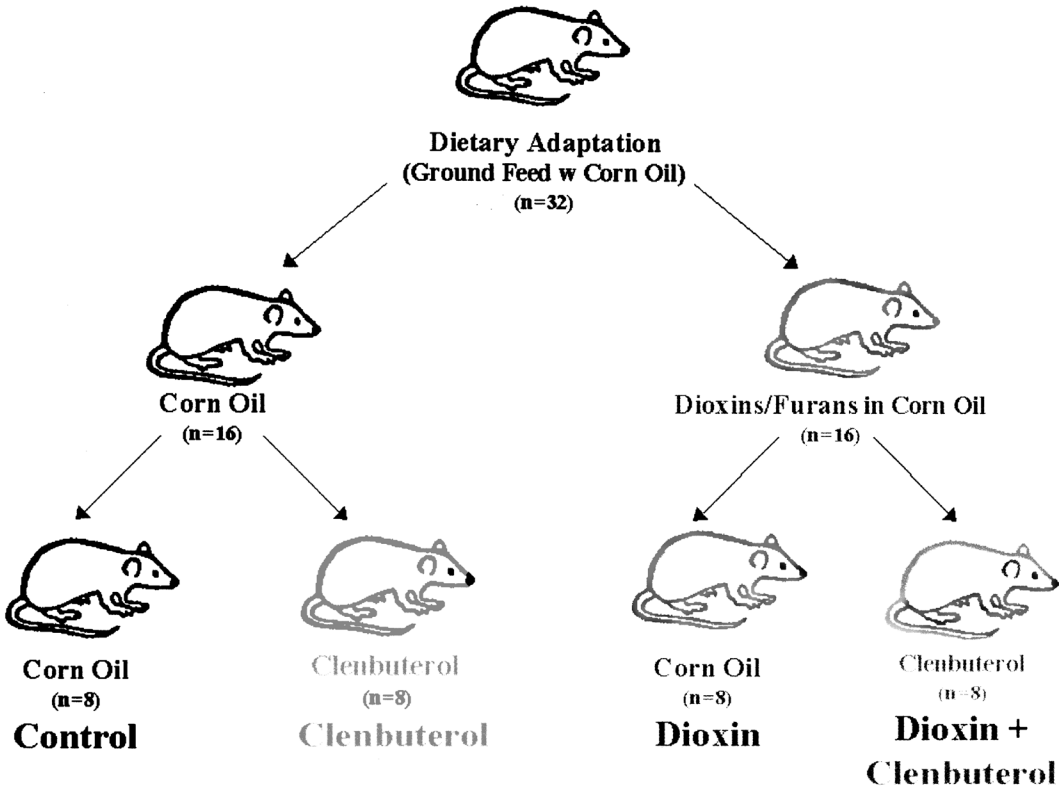


**Figure 1**. Experimental design.

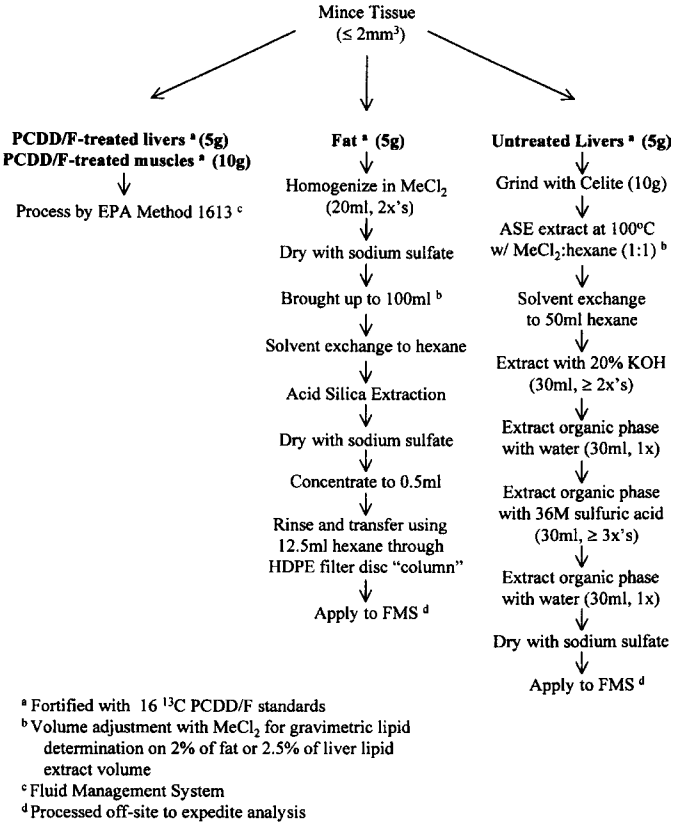


**Figure 2**. Schematic of tissue extractions for polychlorinated dioxin and furan (PCDD/F).

SAS Institute, Cary, NC) with dioxin-dosed and non-dosed groups analyzed separately. This was done because those rats never receiving exogenously dosed PDD/F (control and clenbuterol rats) have extremely low concentrations of PCDD/F; therefore, combining analyses with dioxin-dosed groups was inappropriate. Clenbuterol treatment was the independent variable and replicate was used as a blocking factor. Other parameters were tested using a two-way mixed model analysis of variance. All statistical analyses were performed on untransformed numerical data with the exception of liver, testes, kidneys, and lung weights, which were analyzed on an absolute basis and as a percentage of BW minus gastrointestinal tract weight. For ease of interpretation, data are often presented as percentages. The software program that allows for three-dimensional presentation (Sigma Plot, Version 7.01, SPSS Inc., Chicago, IL) did not allow for error bars; therefore, tables should be consulted for variance data.

## Results and Discussion

### *Physiological Responses to Clenbuterol Treatment*

In spite of randomizing treatment assignment within groups, control rats consumed slightly less feed ($P < 0.03$; ~4%) than rats assigned to the dioxin treatment (both on d 0, prior to treatment, and on d 8 and 9, Table 2). The planned experimental design called for 10 d of clenbuterol treatment followed by transport to

**Table 1**. Doses of polychlorinated dioxins and furans and their respective toxic equivalency factors (TEF; NATO/CCMS, 1988)

| Item | Theoretical dose[a] (in 10 d) | Actual dose[b] (in 10 d) | TEF |
|---|---|---|---|
| Congener | nanograms | | |
| 2,3,7,8 TCDF | 6 | 7.1 | 0.1 |
| 2,3,4,7,8 PeCDF | 10 | 9.8 | 0.5 |
| 1,2,3,4,6,7,8 HpCDF | 6 | 7.8 | 0.01 |
| OCDF | 27 | 28.0 | 0.001 |
| 2,3,7,8 TCDD | 3 | 3.6 | 1.0 |
| 1,2,3,7,8 PeCDD | 3 | 3.6 | 0.5 |
| 1,2,3,6,7,8 H×CDD | 13 | 16.6 | 0.1 |
| 1,2,3,4,6,7,8 HpCDD | 27 | 26.1 | 0.01 |
| OCDD | 27 | 24.1 | 0.001 |
| Non-dosed congener[d] | | | |
| 1,2,3,7,8 PeCDF | | BLQ[c] | 0.05 |
| 1,2,3,4,7,8 H×CDF | | 0.016 | 0.1 |
| 1,2,3,6,7,8 H×CDF | | 0.014 | 0.1 |
| 2,3,4,6,7,8 H×CDF | | 0.152 | 0.1 |
| 1,2,3,7,8,9 H×CDF | | 0.004 | 0.1 |
| 1,2,3,4,7,8,9 HpCDF | | 0.042 | 0.01 |
| 1,2,3,4,7,8 H×CDD | | 0.021 | 0.1 |
| 1,2,3,7,8,9 H×CDD | | 0.383 | 0.1 |

[a]Based on calculated concentrations.
[b]From analyses of dosing solution.
[c]Below limits of quantitation.
[d]Congeners analyzed for, but not in "dose."

Grand Forks, anesthetization, and determination of body composition using a DEXA (dual-energy X-ray absorptometry) densitometer. The instrument broke down the first day and it was concluded (after several days) that timely repairs were not possible. The clenbuterol feeding period had been extended to 16 d. Feed intake data was invalid for the transported group (one replicate); therefore, the last data points used were pretransport. The two animals that received anesthesia were excluded from PCDD/F tissue analysis (still maintaining a sample size of four) but were included in all other data sets (tissue and feeding data, sample size of eight). Feed intake on d 19 to 21 was not different among groups. Clenbuterol improved feed efficiency ($P < 0.002$) and increased body weight ($P < 0.002$; Figure 3). In 10 d of feeding clenbuterol, feed efficiency was improved by 45%; control and dioxin treated rats consumed 8.2 ± 0.37 g of feed per gram of weight gain vs 4.5 ± 0.37 g for clenbuterol and dioxin plus clenbuterol-treated rats (LSM ± S.E., ($P \leq 0.0001$). Similar increased feed efficiency was reported for clenbuterol treatment of female rats (Perez-Llamas and Zamora, 1991). Body weight increased 8% with clenbuterol treatment (305 g vs 327 g; $P \leq 0.002$), which agrees with previous literature (Sillence et al., 1991).

Clenbuterol produced most of the phenotypic changes previously reported in rats (Figure 4; Reeds et al., 1988; Carter et al., 1991; Cartana et al., 1994). With clenbuterol treatment, muscle weight increased 25%, regardless of PCDD/F exposure ($P \leq 0.0001$; Figure 4), and

**Table 2**. Rat feed intake during experimental periods: d 0, d 8 to 9 (dioxin dosing), and d 19 to 20 (clenbuterol feeding)[a]

| Treatment[b] | Day 0[c] | Day 8–9[d] | Day 19–20[e] |
|---|---|---|---|
| Control | 0.079 (±0.0009) | 0.072 (±0.0008) | 0.069 (±0.0015) |
| Dioxin | 0.082[f] | 0.075[f] | 0.068 |
| Clenbuterol | | | 0.066 |
| Dioxin + clenbuterol | | 0.066 | |

[a]Data are expressed as least square means (g/[d·kg]) ± pooled S.E.
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[c]n = 16.
[d]n = 16.
[e]n = 8.
[f]Different from control, $P \leq 0.03$.

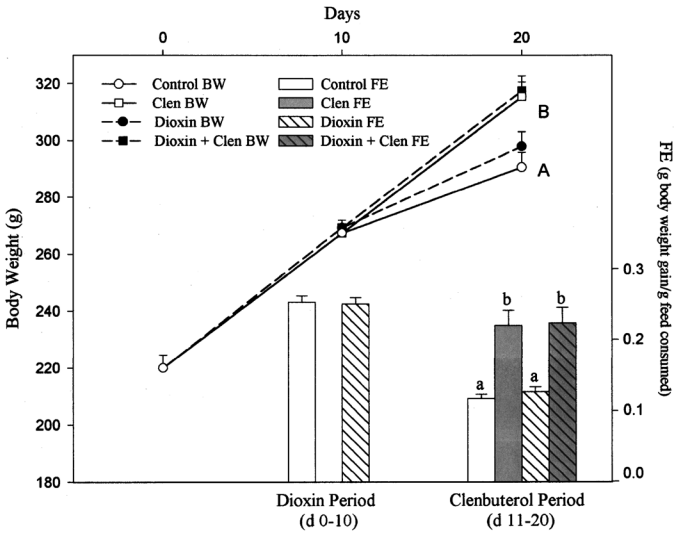


**Figure 3**. Clenbuterol effect on body weight (BW) and feed efficiency (FE). Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture, followed by clenbuterol (dioxin + clenbuterol). Graphed lines represent changes in body weight (BW), bars represent FE over two time periods (LSM ± pooled S.E.). Animal number for d 0 is 32, d 10 is 16, and d 20 is 8. Points or bars with differing letters indicate clenbuterol effects ($P \leq 0.002$).

fat decreased by ~23% ($P \leq 0.0007$; Figure 5). Dioxin treatment, when not followed by clenbuterol, increased fat weight by 19% (Figure 5; $P \leq 0.06$). This finding is of particular interest for two reasons. First, these results are in contrast to the one commonly reported symptom of PCDD/F toxicity—the wasting syndrome. Despite the depth of research on PCDD/F, the mechanism of toxicity and the wasting syndrome are not completely understood (Pohjanvirta and Tuomisto, 1994). The second reason for interest is that these results were also seen in the preliminary experiment. As for reasons why our rats responded to PCDD/F with increased fat instead of wasting, the most likely explanation would be the low dose used. Wasting syndrome is associated with TCDD doses often more than a hundredfold higher than used here. In addition, the rats had 16-d recovery period in the absence of dioxin feeding, and although no increase in feed intake per gram of BW was observed for this group during this period, the trend for higher BW on d 20 (Figure 3) in the PCDD/F-dosed rats correlated to the higher body fat found at necropsy.

Because animals were killed throughout the day, gastrointestinal (GI) tract contents varied among animals. Therefore, when comparing organ weight on a BW basis, the weight of the GI tract was first subtracted from BW. Liver, testes, kidney and lung weights, as a percentage of BW minus GI, were all reduced approximately 10% by clenbuterol treatment ($P \leq 0.001$, 0.002, 0.002, and 0.02, respectively, Figure 4). Reeds et al.

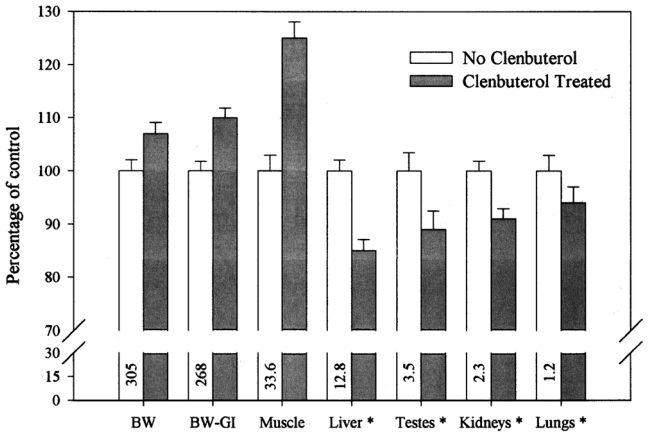


**Figure 4**. Clenbuterol effect on tissue/organ weights. Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture, followed by clenbuterol (dioxin + clenbuterol). The PCDD/F were without effect on these tissues and organs, therefore control- and PCDD/F-treated rats vs clenbuterol- and PCDD/F/clenbuterol-treated rats are presented, the former set normalized to 100% for graphical comparison. Data are LSM ± pooled S.E., n = 8 per treatment, 16 as combined in graph. All tissue weights from clenbuterol treatment were different ($P \leq 0.02$). Values on bars represent absolute weight in grams. *Indicate analysis as % BW − gastrointestinal tract weight.

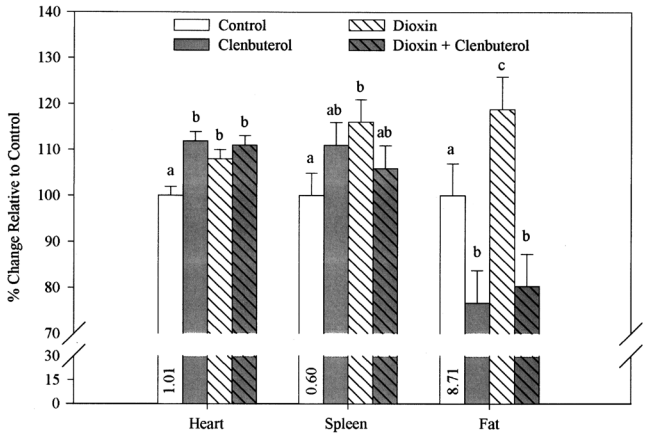


**Figure 5**. Clenbuterol and polychlorinated dioxin and furan effects on organ/tissue weights. Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture, followed by clenbuterol (dioxin + clenbuterol). Values were normalized relative to control (100%), with absolute weight in grams indicated on bars. Data are LSM ± pooled S.E., n = 8. Treatment interactions were found for heart and spleen ($P \leq 0.06$), while the effect of PCDD/F and clenbuterol had the opposite effect on fat weights when given independently. Values with different letters are different ($P \leq 0.05$).

(1988) also reported a decrease in liver weight of rats treated with clenbuterol and two other beta-adrenergic agonists (terbutaline and reproterol). While the effects of clenbuterol on lung and testes weight have not been previously reported, the decrease in kidney weight seen has been reported by both Carter et al. (1991) and Sillence et al. (1991). The explanation for these findings was agreed upon by both Reeds et al. (1988) and Carter et al. (1991); that is, visceral organs are not responsive to the anabolic effect of clenbuterol, only cardiac and skeletal muscles are. Therefore, as muscle mass increases with clenbuterol treatment, the relative weights of the visceral organs decrease. The one exception we found to this was the spleen, which did not decrease with clenbuterol treatment. Another confusing result was that although PCDD/F treatment by itself was without effect on kidney weight (as a percentage of BW), it did ameliorate the decrease in kidney weight observed with clenbuterol treatment (data not shown, 5% decrease vs 12%, $P \leq 0.03$). Heart and spleen organ weights exhibited treatment interactions (Figure 5, $P \leq 0.05$ and 0.06, respectively). Treatment with PCDD/F and clenbuterol, alone or in combination, resulted in increased heart weights (~10%, $P \leq 0.002$ to 0.03). The increase in heart weight with clenbuterol treatment is expected, and was previously reported, as a result of the anabolic effect on the cardiac muscle (Reeds et al., 1988; Sillence et al., 1991). Spleen weight increased with dioxin treatment ($P \leq 0.05$). The literature does not report an increase in heart or spleen wet weight with PCDD/F dosing, but this may reflect lack of observation in chronic low concentration studies (typically evaluating P450 enzyme induction) or a potential compensatory response by the rats, as dosing had been terminated for 16 d.

### *Tissue Results*

*Absorption and Retention of PCDD/F.* To compare absorption and tissue retention among various congeners, data were plotted as a percentage of the dose administered. Absolute values (i.e., masses) of congeners are not presented because concentrations of congeners dosed were not equal (Figure 6). As chlorination

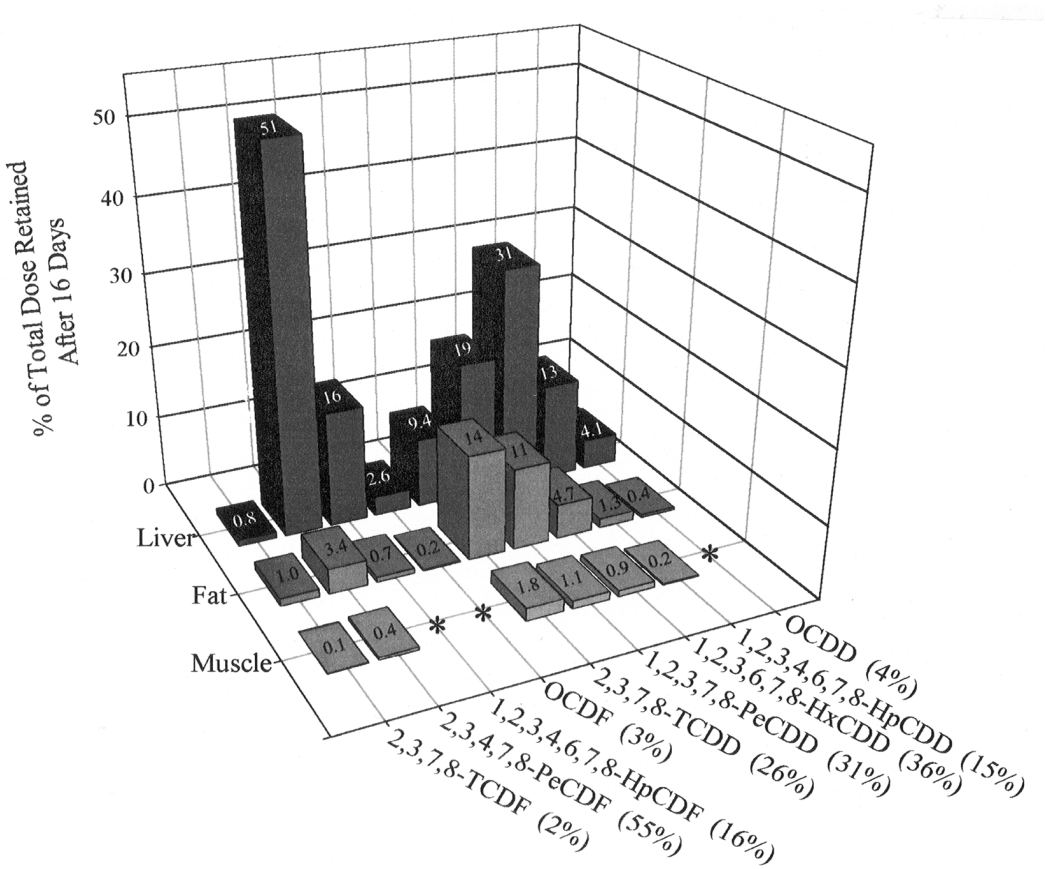


**Figure 6**. Percent of polychlorinated dioxin and furan (PCDD/F) dose retained in tissues. The percent of congener dose found in rat liver, fat and muscle after 16 d of clenbuterol treatment are presented to assess relative tissue distribution and congener accumulation/elimination. Data are LSM, n = 4, pooled S.E. were typically ~8% (software does not allow for additon of S.E. to three dimensional [3-D] plot). Values on bars represent % control, as 3-D graph allows for relational comparison, but skewing of axis (required to see all congeners) leads to inaccuracies of scale. The cumulative dose retained by these tissues are represented by the percentage values next to congener labels.

**Table 3**. Lipid analysis of tissues[a]

| Treatment[b] | Fat | Liver | Muscle |
|---|---|---|---|
| Control | 72.3 ± 2.78 | 2.0 ± 0.12[x] | NA |
| Clenbuterol | 68.1 | 2.5[y] | NA |
| Dioxin | 69.5 | 3.2[z] | 3.0 ± 0.15[y] |
| Dioxin + clenbuterol | 68.2 | 3.1[z] | 1.9[z] |

[a]Based on gravimetric determination of methylene chloride-extractable lipid (% of wet weight). Values are means ± pooled S.E., differing as indicated by superscript x, y, or z ($P \leq 0.02$ for liver control vs clenbuterol, all others $P \leq 0.005$).
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan (PCDD/F) mixture, or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol), n = 4.

number increased past six, congener absorption or retention was decreased as expected (~15% of dose for HpCDD/F down to ~3% for OCDD/F). Literature supporting these findings include Birnbaum and Couture, 1988; McLachlan et al., 1990; and Van den Berg et al., 1987. Retention of penta- and hexa-chlorinated congeners ranged from 31 to 55%, similar to the results obtained in rats 7 d after a single oral dose of the causal rice oil of Yusho disease (Morita et al., 1993). Tetrachlorodibenzo-*p*-dioxin was retained at 26% of dose, whereas very little TCDF (2% of dose) was retained. Liver was the predominant reservoir for most PCDD/F, whereas muscle was the lowest. Fat was intermediate to liver and muscle with the exception of TCDD, of which 14% was contained in the fat and only 9% of the dose in the liver. Liver TCDD results are comparable to those of Wirsling et al. (1996), who found 23% of TCDD dose in rat liver 10 d after dosing (a single oral gavage of 8 ng/kg BW), with retention falling to 3% by d 21. Pentachlorodibenzo-*p*-dioxin was the only other congener present within the same order of magnitude in fat as in liver (11% vs 19%). Relative ranking of TCDD, PeCDD, and H×CDD retention were reversed for liver and fat. In liver, relative retention of these congeners increased as chlorine number increased, but the reverse was true for fat. Similar to reports by Brewster and Birnbaum (1987), PeCDF was approximately an order of magnitude higher in liver vs fat vs muscle. Liver OCDD burden was 10-fold higher than the fat burden, in agreement with data published by Birnbaum and Couture (1988).

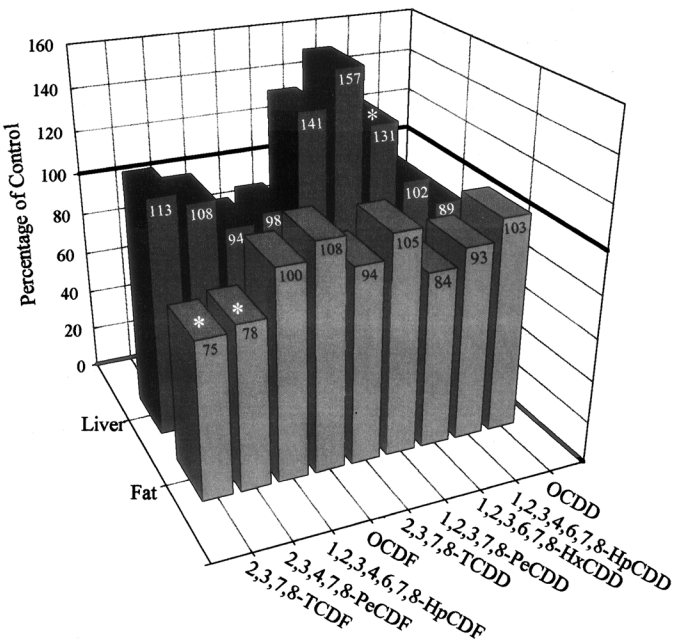


**Figure 7**. Effect of clenbuterol on endogenous polychlorinated dioxin and furan (PCDD/F) total tissue burden. The relative PCDD/F burden (total picograms) of clenbuterol-treated rats are presented, with control values set equal to 100%. Muscle was not analyzed as values would have been below the limits of detection. Data are LSM, n = 4. *Indicates clenbuterol effect ($P \leq 0.03$). Values on bars represent % control group, as three dimensional graph allows for relational comparison, but skewing of axis (required to see all congeners) leads to inaccuracies of scale. See Tables 3–5 for pooled S.E.

*Lipid and Wet Weight Basis.* Results were also expressed on three weight bases: picograms per gram of lipid, picograms per gram of wet weight tissue, and picograms in total tissue (Tables 4, 5, and 6). In the past, due to a high affinity of PCDD/F for fats, congener concentrations were reported on a gram-of-lipid basis as a means of normalization. For the purposes of effective remediation, concentrations need to be considered per gram of wet weight and on a total tissue-burden basis. Before examining the PCDD/F per gram of lipid, first consider potential changes in lipid profiles in response to clenbuterol and their subsequent effect on congener concentrations per gram of lipid. Clenbuterol could 1) have no effect on lipid, 2) decrease lipid, or 3) increase lipid concentrations. If no effect was seen and metabolism of congeners is not occuring, then congener concentrations would remain unchanged. If lipid concentration was decreased without congener metabolism, then congener concentration would increase per gram of lipid. And finally, if lipid concentrations increased without congener metabolism, then congener concentrations would decrease per gram of lipid. Scenario 1) can be seen (Table 3) for fat—no change in lipid concentration. This reflects the consistency of fat composition; although the total grams of fat might increase or decrease, the percentage lipid remained relatively constant. Scenario 2), a lipid concentration decrease, was found in

**Table 4**. Effect of clenbuterol on polychlorinated furan and dioxin concentration in fat of rats[a]

| Congener | Unit | Treatment[b] | | | |
|---|---|---|---|---|---|
| | | Control[c] | Clenbuterol[d] | Dioxin | Dioxin + clenbuterol |
| 2,3,7,8-TCDF | pg/g lipid[e] | 1.01 ± 0.094 | 1.08 | 8.53 ± 1.301 | 4.96[z] |
| | pg/g wet[f] | 0.73 ± 0.038 | 0.72 | 6.02 ± 1.022 | 3.40 |
| | pg[g] | 6.96 ± 0.415 | 5.21[y] | 73.98 ± 14.715 | 25.24[z] |
| 2,3,4,7,8-PeCDF | pg/g lipid | 0.35 ± 0.043 | 0.39 | 40.68 ± 1.554 | 38.77 |
| | pg/g wet | 0.25 ± 0.023 | 0.26 | 28.14 ± 0.853 | 26.42 |
| | pg | 2.40 ± 0.120 | 1.88[y] | 333.14 ± 23.082 | 191.47[y] |
| 1,2,3,4,6,7,8,-HpCDF | pg/g lipid | 0.31 ± 0.078 | 0.45 | 6.72 ± 0.563 | 7.76 |
| | pg/g wet | 0.22 ± 0.046 | 0.30 | 4.62 ± 0.274 | 5.28 |
| | pg | 2.09 ± 0.261 | 2.08 | 54.12 ± 3.102 | 38.30[x] |
| OCDF | pg/g lipid | 0.20 ± 0.024 | 0.30[y] | 5.64 ± 0.422 | 6.43 |
| | pg/g wet | 0.14 ± 0.016 | 0.20[y] | 3.89 ± 0.222 | 4.38 |
| | pg | 1.38 ± 0.160 | 1.49 | 45.75 ± 2.683 | 31.77[x] |
| 2,3,7,8-TCDD | pg/g lipid | 0.55 ± 0.124 | 0.75 | 63.77 ± 4.005 | 75.04[z] |
| | pg/g wet | 0.40 ± 0.073 | 0.50 | 43.95 ± 1.802 | 51.10[y] |
| | pg | 3.77 ± 0.389 | 3.53 | 515.60 ± 25.915 | 370.53[y] |
| 1,2,3,7,8-PeCDD | pg/g lipid | 0.73 ± 0.234 | 1.12 | 49.47 ± 3.061 | 56.79 |
| | pg/g wet | 0.53 ± 0.137 | 0.74 | 34.11 ± 1.358 | 38.66[z] |
| | pg | 4.98 ± 0.795 | 5.24 | 400.88 ± 21.113 | 280.15[x] |
| 1,2,3,6,7,8-H×CDD | pg/g lipid | 0.40 ± 0.054 | 0.49 | 97.11 ± 6.484 | 106.59 |
| | pg/g wet | 0.29 ± 0.031 | 0.32 | 66.92 ± 3.162 | 72.59 |
| | pg | 2.76 ± 0.232 | 2.33 | 786.36 ± 43.318 | 525.68[y] |
| 1,2,3,4,6,7,8-HpCDD | pg/g lipid | 0.97 ± 0.126 | 1.28 | 41.89 ± 3.365 | 48.59 |
| | pg/g wet | 0.71 ± 0.061 | 0.85 | 28.83 ± 1.675 | 33.07 |
| | pg | 6.66 ± 0.484 | 6.17 | 338.23 ± 18.608 | 239.64[x] |
| OCDD | pg/g lipid | 3.53 ± 0.432 | 5.14[y] | 11.37 ± 1.068 | 13.10 |
| | pg/g wet | 2.55 ± 0.290 | 3.47[z] | 7.83 ± 0.644 | 8.93 |
| | pg | 24.11 ± 1.919 | 24.82 | 92.38 ± 5.980 | 64.14[y] |

[a]Data are expressed as least square means ± pooled S.E., n = 4.
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan (PCDD/F) mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[c]Control animals received only corn oil so congener concentrations reflect endogenous body burdens.
[d]This group was included to determine clenbuterol's effect on endogenous body burdens.
[e]Concentration expressed on a picogram of analyte per gram of lipid.
[f]Concentration expressed per gram of wet tissue.
[g]Total mass of analyte present in tissue (wet tissue weight basis).
[x]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.01$.
[y]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.05$.
[z]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.10$.

muscle with clenbuterol treatment (37%, $P \leq 0.004$), reflecting a decrease in fat per gram of muscle, as would be expected. The third scenario, a lipid concentration increase, was seen in liver after both clenbuterol (alone 20%, $P \leq 0.02$) and dioxin treatment (± clenbuterol~ 60%, $P \leq 0.002$). In the case of clenbuterol treatment alone, the mass of the liver was reduced by an amount (~15%, Figure 4) similar to the increase in lipid (20%), resulting in a net increase in liver lipid concentration without increased lipid accretion. This was not the case for dioxin-treated rats, in which the lipid increase was much higher (60%) than the decrease in organ weight (no liver weight change occurred with dioxin treatment alone, whereas an ~20% decrease was found when followed by clenbuterol). Therefore, when looking at the fat data in Table 4, and liver data comparing dioxin to dioxin + clenbuterol (Table 5, where the lipid concentration was equivalent for both), whatever trend is seen in the congener concentration on a picogram-per-gram wet basis is consistent with that found in the congener concentration based on picograms per gram of lipid. When comparing control to clenbuterol for liver (Table 5) and dioxin to dioxin + clenbuterol for muscle data (Table 6), wet weight vs lipid basis concentrations should be considered separately, due to differences in percent lipid.

When comparing dioxin to dioxin followed by clenbuterol treatment in fat (Table 4) starting with the third congener listed (1,2,3,4,6,7,8-HpCDF) and continuing through the following six congeners, concentrations on a wet weight or on a lipid basis consistently increased with clenbuterol treatment. This increase was signifi-

**Table 5**. Effect of clenbuterol on polychlorinated furan and dioxin concentration in liver of rats[a]

| Congener | Unit | Treatment[b] | | | |
|---|---|---|---|---|---|
| | | Control | Clenbuterol | Dioxin | Dioxin + clenbuterol |
| 2,3,7,8-TCDF | pg/g lipid[c] | 48.60 ± 23.79 | 47.16 | 135.00 ± 6.0 | 93.00[y] |
| | pg/g wet[d] | 0.93 ± 0.038 | 1.15[y] | 4.26 ± 0.217 | 2.94[y] |
| | pg[e] | 12.40 ± 0.670 | 13.99 | 59.00 ± 3.3 | 36.0[z] |
| 2,3,4,7,8-PeCDF | pg/g lipid | 89.40 ± 8.345 | 84.19 | 11,592 ± 1,201.6 | 12,730 |
| | pg/g wet | 1.71 ± 0.176 | 2.04 | 371.25 ± 48.661 | 400.75 |
| | pg | 23.18 ± 1.465 | 24.97 | 5,018 ± 480.9 | 4,799 |
| 1,2,3,4,6,7,8,-HpCDF | pg/g lipid | 48.92 ± 1.791 | 41.26[z] | 2,835 ± 178.8 | 3,224 |
| | pg/g wet | 0.94 ± 0.032 | 1.00 | 90.80 ± 8.840 | 101.43 |
| | pg | 13.00 ± 1.348 | 12.21 | 1,230 ± 72.3 | 1,213 |
| OCDF | pg/g lipid | 20.04 ± 0.315 | 18.62[y] | 1,725 ± 259.6 | 1,966 |
| | pg/g wet | 0.39 ± 0.016 | 0.46[z] | 55.47 ± 9.359 | 61.72 |
| | pg | 5.56 ± 0.472 | 5.53 | 741.0 ± 106.7 | 744 |
| 2,3,7,8-TCDD | pg/g lipid | 10.62 ± 1.028 | 12.94 | 790.0 ± 133.4 | 1,087 |
| | pg/g wet | 0.20 ± 0.019 | 0.31[y] | 25.52 ± 5.028 | 34.32 |
| | pg | 2.75 ± 0.344 | 3.79 | 340 ± 49.8 | 404 |
| 1,2,3,7,8-PeCDD | pg/g lipid | 32.50 ± 7.823 | 47.46 | 1,572 ± 256.4 | 2,076 |
| | pg/g wet | 0.62 ± 0.152 | 1.13[z] | 50.82 ± 9.988 | 65.52 |
| | pg | 8.66 ± 2.475 | 13.72 | 677 ± 99.3 | 776 |
| 1,2,3,6,7,8-H×CDD | pg/g lipid | 47.10 ± 5.568 | 53.26 | 11,731 ± 1,518.3 | 15,119 |
| | pg/g wet | 0.90 ± 0.130 | 1.30 | 377.50 ± 62.510 | 476.0 |
| | pg | 12.10 ± 0.604 | 15.77[y] | 5,108 ± 512.4 | 5,681 |
| 1,2,3,4,6,7,8-HpCDD | pg/g lipid | 189.57 ± 7.164 | 170.50 | 8,049 ± 988.1 | 8,906 |
| | pg/g wet | 3.64 ± 0.141 | 4.18[z] | 259.00 ± 40.221 | 280.5 |
| | pg | 49.60 ± 4.628 | 50.60 | 3,470 ± 410.6 | 3,372 |
| OCDD | pg/g lipid | 758.16 ± 23.708 | 601.23[y] | 2,266 ± 203.6 | 2,637 |
| | pg/g wet | 14.57 ± 0.508 | 14.75 | 72.58 ± 7.845 | 82.78 |
| | pg | 200.11 ± 21.060 | 178.78 | 982 ± 83.6 | 994 |

[a]Data are expressed as least square means ± pooled S.E., n = 4.
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[c]Concentration expressed on a picogram of analyte per gram of lipid.
[d]Concentration expressed per gram of wet tissue.
[e]Total mass of analyte present in tissue (wet tissue weight basis).
[x]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.01$.
[y]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.05$.
[z]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.10$.

cant for TCDD on a lipid-weight basis (increasing 18%, $P \leq 0.10$) and for TCDD and PeCDD on a wet weight basis (increasing 16%, $P \leq 0.03$, and 13%, $P \leq 0.06$, respectively). The probability of consistency of findings across seven congeners and the small sample number used should be considered before dismissing values without statistical significance.

The same seven congener concentrations tend to increase in liver (on both a lipid and a wet weight basis) when dioxin treatment is followed by clenbuterol (Table 5). While three of these seven congeners are below the limits of quantification in muscle (Table 6), the remaining four exhibit inverse trends when expressed on a lipid vs wet weight. As a result of the decrease in lipid content per gram of wet muscle, the trend is for increases in concentration of exogenous PCDD/F per gram of lipid (22% for 2,3,7,8-TCDD, $P \leq 0.10$; 23% for 1,2,3,7,8-PeCDD, $P \leq 0.10$; 8% for 1,2,3,6,7,8-H×CDD, not significant [NS]; and 20% for 1,2,3,4,6,7,8-HpCDD, NS), whereas on a wet weight basis these congener concentrations decreased (25%, $P \leq 0.05$; 24%, $P \leq 0.05$; 34%, $P \leq 0.01$; and 30%, NS, respectively).

The first two congeners in Tables 4 through 6 do not fit with the trends seen in the other congeners. The first congener, 2,3,7,8-TCDF, decreased at least 30% in all tissues on a picogram-per-gram-of-lipid basis when dioxin treatment was followed by clenbuterol (42%, 49%, and 31% for fat, liver, and muscle, respectively). This is most likely related to the high rate of metabolism associated with 2,3,7,8-TCDF (Birnbaum et al., 1980), an assumption strongly supported by the recovery of only 2% of the dosed TCDF in the three tissues combined (Figure 6). The second congener, PeCDF, showed no concentration change in fat (Table 4) or liver (Table 5) on a lipid or wet weight basis but was decreased in muscle (Table 6, $P \leq 0.01$) on a wet weight basis. The fact that 2,3,4,7,8-PeCDF was the congener with the highest relative retention as percent of dose (Figure 6)

**Table 6**. Effect of clenbuterol on polychlorinated furan and dioxin concentration in muscle of rats[ab]

| Congener | Unit | Treatment[c] Dioxin | Dioxin + clenbuterol |
|---|---|---|---|
| 2,3,7,8-TCDF | pg/g lipid[d] | 5.44 ± 0.700 | 2.79[y] |
| | pg/g wet[e] | 0.16 ± 0.018 | 0.05[x] |
| | pg[f] | 5.78 ± 0.873 | 2.25[y] |
| 2,3,4,7,8-PeCDF | pg/g lipid | 36.91 ± 1.631 | 39.07 |
| | pg/g wet | 1.12 ± 0.034 | 0.73[w] |
| | pg | 39.11 ± 2.487 | 30.84[y] |
| 1,2,3,4,6,7,8,-HpCDF | pg/g lipid | BLQ | BLQ |
| | pg/g wet | BLQ | BLQ |
| | pg | BLQ | BLQ |
| OCDF | pg/g lipid | BLQ | BLQ |
| | pg/g wet | BLQ | BLQ |
| | pg | BLQ | BLQ |
| 2,3,7,8-TCDD | pg/g lipid | 59.35 ± 3.277 | 72.51[y] |
| | pg/g wet | 1.79 ± 0.063 | 1.34[x] |
| | pg | 63.03 ± 3.562 | 56.23 |
| 1,2,3,7,8-PeCDD | pg/g lipid | 36.17 ± 2.308 | 44.38[y] |
| | pg/g wet | 1.09 ± 0.037 | 0.83[x] |
| | pg | 38.14 ± 0.995 | 34.97[z] |
| 1,2,3,6,7,8-H×CDD | pg/g lipid | 132.32 ± 6.816 | 142.75 |
| | pg/g wet | 4.01 ± 0.174 | 2.64[w] |
| | pg | 141.42 ± 9.472 | 111.35[z] |
| 1,2,3,4,6,7,8-HpCDD | pg/g lipid | 41.14 ± 9.481 | 49.31 |
| | pg/g wet | 1.26 ± 0.191 | 0.88 |
| | pg | 44.29 ± 7.603 | 37.01 |
| OCDD | pg/g lipid | BLQ | BLQ |
| | pg/g wet | BLQ | BLQ |
| | pg | BLQ | BLQ |

[a]Data are expressed as least square means ± pooled S.E., n = 4.
[b]Muscle from control and clenbuterol treated rats were not analyzed as concentrations were below limits of quantitation (BLQ).
[c]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[d]Concentration expressed on a picogram of analyte per gram of lipid.
[e]Concentration expressed per gram of wet tissue.
[f]Total mass of analyte present in tissue (wet tissue weight basis).
[W]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.01$.
[X]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.05$.
[Y]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.10$.
[Z]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.11$.

is consistent with its persistence in fat and liver and in agreement with its reported half-life of 193 d in Fischer 344 rats (Brewster and Birnbaum, 1987) vs the 1-d half-life of 2,3,7,8-TCDF in liver (Birnbaum et al., 1980).

Clenbuterol's effect on endogenous congener concentrations in fat is also presented in Table 4. The concentration of congeners in fat from rats not dosed with PCDD/F responded to clenbuterol in the same way as those dosed with PCDD/F. That is, the third through ninth congeners tended to increase on both a wet and lipid weight basis after clenbuterol treatment, with significance achieved in the case of two congeners (OCDF 43%, $P \leq 0.04$, and OCDD 36%, $P \leq 0.07$). Unlike after PCDD/F dosing, clenbuterol alone did not decrease the concentrations of TCDF, but, similar to PCDD/F-dosed rats, PeCDF remained unchanged.

Congener concentrations from liver of rats receiving only clenbuterol are less consistent than those from rats treated with dioxin followed by clenbuterol (Table 5). Small but significant decreases in congener concentration on a lipid weight basis were seen with both HpCDF (16%, $P \leq 0.10$) and OCDF (7%, $P \leq 0.05$). These decreases are in contrast to increases seen with dioxin followed by clenbuterol treatment. On a wet weight basis, concentrations of OCDF, TCDD, PeCDD, and HpCDD all increased in rats treated only with clenbuterol (15 to 82%, $P \leq 0.03$ to 0.10), which are similar to trends seen with dioxin followed by clenbuterol. Increases were somewhat greater than would be expected by the 7% decrease in liver weight associated with clenbuterol treatment. Perhaps of note, one congener, 1,2,3,4,7,8,9-HpCDF, tended to decrease in rats without exogenous treatment in both concentration (16%, wet

**Table 7**. Effect of clenbuterol on "non-dosed congener" concentrations in fat[a]

| Congener | Unit | Treatment[b] | | | |
|---|---|---|---|---|---|
| | | Control | Clenbuterol | Dioxin | Dioxin + clenbuterol |
| 1,2,3,7,8-PeCDF | pg/g lipid[c] | 0.245 ± 0.0491 | 0.299 | 0.213 ± 0.0395 | 0.141 |
| | pg/g wet[d] | 0.177 ± 0.0280 | 0.198[z] | 0.151 ± 0.0310 | 0.097 |
| | pg[e] | 1.677 ± 0.1827 | 1.422 | 1.816 ± 0.3735 | 0.695 |
| 1,2,3,4,7,8-H×CDF | pg/g lipid | 0.361 ± 0.1152 | 0.532 | 0.480 ± 0.0782 | 0.448 |
| | pg/g wet | 0.260 ± 0.0673 | 0.348 | 0.336 ± 0.0597 | 0.305 |
| | pg | 2.472 ± 0.4134 | 2.462 | 3.949 ± 0.6650 | 2.210 |
| 1,2,3,6,7,8,-H×CDF | pg/g lipid | 0.200 ± 0.0337 | 0.223 | 0.221 ± 0.0359 | 0.225 |
| | pg/g wet | 0.144 ± 0.0207 | 0.149 | 0.153 ± 0.0254 | 0.153 |
| | pg | 1.367 ± 0.1388 | 1.058 | 1.779 ± 0.2634 | 1.107 |
| 2,3,4,6,7,8,-H×CDF | pg/g lipid | 0.153 ± 0.0223 | 0.201 | 0.552 ± 0.0411 | 0.550 |
| | pg/g wet | 0.111 ± 0.0135 | 0.135 | 0.383 ± 0.0290 | 0.374 |
| | pg | 1.044 ± 0.1364 | 0.992 | 4.505 ± 0.3732 | 2.719[x] |
| 1,2,3,7,8,9-H×CDF | pg/g lipid | 0.032 ± 0.0182 | 0.040 | 0.025 ± 0.0200 | 0.066 |
| | pg/g wet | 0.022 ± 0.0121 | 0.026 | 0.019 ± 0.0145 | 0.045 |
| | pg | 0.218 ± 0.1074 | 0.206 | 0.216 ± 0.1564 | 0.317 |
| 1,2,3,4,7,8,9-HpCDF | pg/g lipid | 0.031 ± 0.0180 | 0.095[y] | 0.080 ± 0.0224 | 0.107 |
| | pg/g wet | 0.022 ± 0.0116 | 0.063[y] | 0.056 ± 0.0162 | 0.073 |
| | pg | 0.208 ± 0.1144 | 0.469 | 0.705 ± 0.2030 | 0.521 |
| 1,2,3,4,7,8-H×CDD | pg/g lipid | 0.178 ± 0.0237 | 0.174 | 0.297 ± 0.0198 | 0.309 |
| | pg/g wet | 0.128 ± 0.0131 | 0.115 | 0.206 ± 0.0159 | 0.211 |
| | pg | 1.219 ± 0.0905 | 0.824[y] | 2.440 ± 0.2215 | 1.522[z] |
| 1,2,3,7,8,9-H×CDD | pg/g lipid | 0.168 ± 0.0240 | 0.189 | 1.276 ± 0.1214 | 1.148 |
| | pg/g wet | 0.122 ± 0.0142 | 0.125 | 0.880 ± 0.0753 | 0.967 |
| | pg | 1.152 ± 0.1412 | 0.915 | 10.466 ± 1.0449 | 6.991[z] |

[a]Data are expressed as least square means ± pooled S.E., n = 4.
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[c]Concentration expressed on a picogram of analyte per gram of lipid.
[d]Concentration expressed per gram of wet tissue.
[e]Total mass of analyte present in tissue (wet tissue weight basis).
[x]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.01$.
[y]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.05$.
[z]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.10$.

weight) and total pg (34%) (Table 7); this same congener in fat increased wet weight concentration 186% ($P \leq 0.05$, Table 6). Although liver weights were lower and percent lipid in liver was higher for clenbuterol-treated than control rats, it is not clear why some congeners responded in an inverse manner when compared on a wet vs lipid weight basis.

Concentrations of non-dosed congeners are given in Tables 7 (fat) and 8 (liver). When interpreting these data it should be remembered that these concentrations are extremely low, causing inherent inaccuracies in quantitation. Muscle data is not reported as no values were above the limits of quantitation. Clenbuterol treatment after dioxin dosing caused no significant changes in congener concentrations in fat. Clenbuterol treatment without dioxin dosing resulted in a 12% increase of 1,2,3,7,8-PeCDF on a weight wet basis ($P \leq 0.10$), and 1,2,3,4,7,8,9-HpCDF increased ~200% on both a wet and lipid weight basis ($P \leq 0.05$, Table 7). Concentrations in liver (Table 8) were unchanged by all treatments with the exception of the effect of clenbuterol alone on the H×CDD (1,2,3,4,7,8-H×CDD and 1,2,3,7,8,9-H×CDD) which increased ~29% on a wet weight basis ($P \leq 0.05$).

### *Total Picograms per Tissue— Indicator of Remediation Potential*

*Fat.* Clenbuterol treatment decreased the endogenous burden (total picograms) in fat of TCDF and PeCDF (≥22%, $P < 0.02$, Table 4 and Figure 7) and 1,2,3,4,7,8-H×CDD (32%, $P < 0.02$, Table 7). It is possible that clenbuterol may have reduced the burdens of other congeners, but the fact that non-dosed congener levels were close to the limits of quantitation may have prevented such assessment.

In rats dosed with PCDD/F, clenbuterol was effective in reducing burdens of every congener in fat (total picograms) by ~30% with the exception of PeCDF and TCDF, which were reduced by 43% and 66%, respectively (Figure 8, Table 4). This reduction in fat burden (≥30% of total pg) was a trend also found in the non-dosed congeners for all but one congener, 1,2,3,7,8,9-H×CDF (Table 7). Caution should be taken when con-

**Table 8**. Effect of clenbuterol on "non-dosed congener" concentrations in liver[a]

| Congener | Unit | Treatment[b] Control | Clenbuterol | Dioxin | Dioxin + clenbuterol |
|---|---|---|---|---|---|
| 1,2,3,7,8-PeCDF | pg/g lipid[c] | 9.95 ± 0.316 | 9.712 | BLQ | BLQ |
| | pg/g wet[d] | 0.201 ± 0.0103 | 0.238 | BLQ | BLQ |
| | pg[e] | 2.877 ± 0.3387 | 2.903 | BLQ | BLQ |
| 1,2,3,4,7,8-H×CDF | pg/g lipid | 42.21 ± 3.087 | 40.55 | 35.43 ± 3.502 | 44.93 |
| | pg/g wet | 0.912 ± 0.0565 | 1.005 | 1.146 ± 0.4427 | 1.413 |
| | pg | 12.949 ± 1.9838 | 12.64 | 15.01 ± 5.071 | 16.53 |
| 1,2,3,6,7,8-H×CDF | pg/g lipid | 27.18 ± 2.269 | 23.55 | 30.61 ± 3.555 | 31.11 |
| | pg/g wet | 0.547 ± 0.0472 | 0.572 | 0.986 ± 0.1241 | 0.979 |
| | pg | 7.817 ± 1.5420 | 6.985 | 13.12 ± 1.127 | 11.18 |
| 2,3,4,6,7,8-H×CDF | pg/g lipid | 24.05 ± 2.864 | 20.33 | 119.65 ± 7.878 | 133.13 |
| | pg/g wet | 0.489 ± 0.0599 | 0.494 | 3.830 ± 0.3765 | 4.193 |
| | pg | 7.001 ± 1.5222 | 6.045 | 51.65 ± 4.074 | 50.04 |
| 1,2,3,7,8,9-H×CDF | pg/g lipid | 2.07 ± 1.3668 | 2.445 | BLQ | BLQ |
| | pg/g wet | 0.038 ± 0.0286 | 0.061 | BLQ | BLQ |
| | pg | 0.715 ± 0.5221 | 0.705 | BLQ | BLQ |
| 1,2,3,4,7,8,9-HpCDF | pg/g lipid | 5.811 ± 1.4964 | 4.197 | BLQ | BLQ |
| | pg/g wet | 0.121 ± 0.0308 | 0.101 | BLQ | BLQ |
| | pg | 1.895 ± 0.5905 | 1.255 | BLQ | BLQ |
| 1,2,3,4,7,8-H×CDD | pg/g lipid | 11.75 ± 0.364 | 12.29 | BLQ | BLQ |
| | pg/g wet | 0.235 ± 0.0091 | 0.302[z] | BLQ | BLQ |
| | pg | 3.325 ± 0.3914 | 3.659 | BLQ | BLQ |
| 1,2,3,7,8,9-H×CDD | pg/g lipid | 18.39 ± 0.715 | 19.38 | 300.50 ± 50.272 | 329.94 |
| | pg/g wet | 0.369 ± 0.0190 | 0.478[z] | 9.675 ± 1.8435 | 10.385 |
| | pg | 5.231 ± 0.7393 | 5.765 | 129.44 ± 19.274 | 124.05 |

[a]Data are expressed as least square means ± pooled S.E., n = 4.
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[c]Concentration expressed on a picogram of analyte per gram of lipid.
[d]Concentration expressed per gram of wet tissue.
[e]Total mass of analyte present in tissue (wet tissue weight basis).
[z]Means differ (control vs clenbuterol or dioxin vs dioxin + clenbuterol) $P \leq 0.05$.

sidering this congener, as its concentration was very low, appearing to increase 47% in response to clenbuterol. Total picograms of the following congeners were reduced by clenbuterol treatment: 2,3,4,6,7,8-H×CDF (40%, $P < 0.01$); 1,2,3,4,7,8-H×CDD (32%, $P < 0.02$); and 1,2,3,7,8,9-H×CDD (33%, $P < 0.08$). So while the concentration of most dosed PCDD/F increased in the fat (due to a decrease in the amount of fat), total body burdens in the fat decreased. The smaller reduction in fat body burden in rats not dosed with exogenous PCDD/F might be a reflection of differences in the mobilization capacity of congeners that have been stored in the fat for a short vs a long period of time. The non-dosed congener data would appear to support this hypothesis, as these congeners, which also showed lower reductions with clenbuterol treatment, were present at the beginning of the experimental period and were presumably acquired perinatally from the dam and not acquired only 2 wk prior to clenbuterol treatment. An alternative explanation is that autoinduction of dioxin-metabolizing enzymes occurred in the PCDD/F-dosed rats, reflected by the greater relative depletion of 2,3,7,8-TCDF from fat in these rats following clenbuterol treatment (66%, Figure 8) compared to rats that received clenbuterol alone (25%, Figure 7). Literature reports for induction of cytochromes P450 1A1 and 1A2 by PCDD/F, with a concomitant increase in PCDD/F metabolism, has been reported for rats in vitro and in vivo (Olson et al., 1991; McKinley et al., 1991).

*Liver.* On a total liver basis, TCDF was the only congener to be significantly reduced by clenbuterol treatment in PCDD/F-treated animals (39% reduction, $P \leq 0.06$, Figure 8, Table 5). As discussed above, this is in agreement with TCDF's high capacity to be metabolized and may reflect autoinduction of liver enzymes because TCDF was not reduced in the liver of rats treated only with clenbuterol. Of the dioxin congeners (not furan), 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, and 1,2,3,4,6,7,8-HpCDD tended to increase in liver after clenbuterol treatment, but the increase was greater without exogenous PCDD/F dosing (31 to 57% vs 11 to 19%). The net effect of clenbuterol treatment on the liver was a slight increase in total burden (picograms) of PCDD/F.

*Muscle.* The total burden (picograms) of all six quantifiable dosed congeners in muscle tended to be reduced with clenbuterol treatment (Figure 8 and Table 6). Percent reductions ranged from 8% (1,2,3,7,8-PeCDD, $P \leq 0.11$) to 61% for 2,3,7,8-TCDF ($P \leq 0.10$). Although

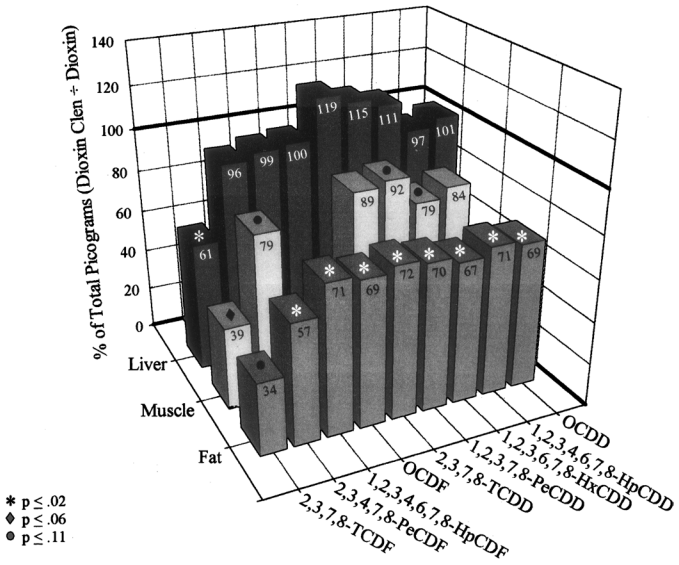


**Figure 8**. Effect of clenbuterol on polychlorinated dioxin and furan (PCDD/F) total tissue burden after exogenous PCDD/F exposure. The relative PCDD/F burden (total picograms) after clenbuterol treatment are presented with dioxin values set equal to 100%. Data are LSM, n = 4, symbols indicate clenbuterol effect with corresponding *P*-value. HpCDF, OCDF, and OCDD were below the limits of quantitation in muscle. Values on bars represent % dioxin burden, as three dimensional graph allows for relational comparison, but skewing of axis (required to see all congeners) leads to inaccuracies of scale. See Tables 3–5 for pooled S.E.

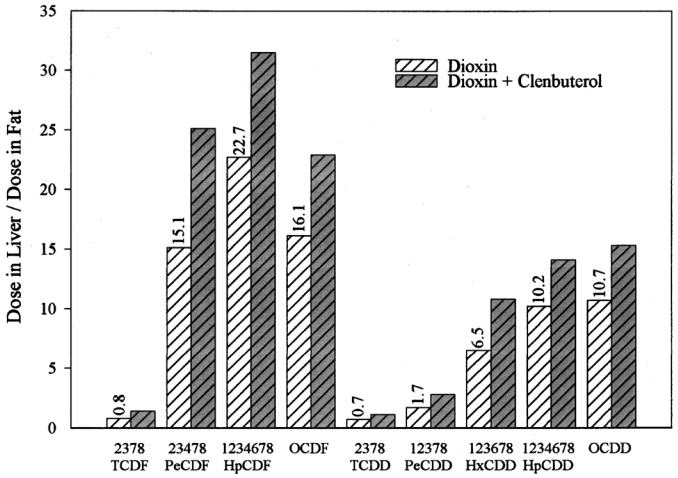


**Figure 9**. Effect of clenbuterol on liver to fat ratio of polychlorinated dioxins and furans (PCDD/F). The percent of the PCDD/F dose found in the liver was divided by percent in the fat to indicate relative PCDD/F distribution. Clenbuterol shifted the ratio toward the liver in all congeners (n = 4). These ratios reflect an inherent overestimation, as total fat is based on grams dissected, not total body fat.

2,3,7,8-TCDD and 1,2,3,4,6,7,8-HpCDD were not statistically different after clenbuterol treatment, the total grams were 11% and 16% lower. Again, as found in fat and liver, 2,3,7,8-TCDF exhibited the greatest total reduction. Non-dosed congeners were not present in adequate concentrations to be quantitated. PCDD/F burdens in muscle were lowered by clenbuterol treatment, although not to as great of an extent as in fat.

*Fat to Liver Ratios and TEQ*. One way to assess shifts in the body burdens of PCDD/F is by measuring the ratio of PCDD/F in liver and fat (Figure 9). The data from our dioxin-dosed rats are consistent with those found in rats, marmosets, and humans, as summarized by Van den Berg et al. (1994), and indicate that hepatic PCDD/F deposition increases as chlorination number increases. Clenbuterol treatment shifted PCDD/F burdens in the direction of the liver (increasing hepatic concentrations from 38 to 75%, Figure 9). Such a shift could be an advantage for remediation purposes. By

**Table 9**. Tissue toxic equivalency quotients (TEQ) and distributions of TEQs among tissues for clenbuterol- and dioxin-treated rats[a]

| | Treatment[b] | | | | | | Relative clenbuterol change | |
|---|---|---|---|---|---|---|---|---|
| | | | Dioxin | | Dioxin + clenbuterol | | | |
| Item | Control TEQ (pg) | Clenbuterol TEQ (pg) | TEQ (pg) | Fraction (%) | TEQ (pg) | Fraction (%) | Dioxin[c] (%) | Control[d] (%) |
| Fat | 10.1 ± 1.10 | 9.3 | 976 ± 63.86 | 20 | 666 | 15 | –32[z] | –8 |
| Muscle | NA[e] | NA | 117 | 2 | 101 | 2 | –14 | NA |
| Liver | 28.0 ± 3.38 | 31.9 | 3,752 ± 368.2 | 78 | 3,811 | 83 | No change | 14 |

[a]Data are expressed as least square means ± pooled S.E., n = 4.
[b]Rats were either treated with corn oil (control), clenbuterol alone (clenbuterol), a polychlorinated dioxin and furan [PCDD/F] mixture (dioxin), or a PCDD/F mixture followed by clenbuterol (dioxin + clenbuterol).
[c]Relative change within a tissue was calculated by dividing the TEQ for the dioxin + clenbuterol treatment by the TEQ value for dioxin treatment.
[d]Relative change within a tissue was calculated by dividing the TEQ for the clenbuterol treatment by the TEQ value for the control treatment.
[e]NA, Not analyzed; muscles from control and clenbuterol treated rats would have PCDD/F levels below the limit of quantitation.
[z]TEQ decreased significantly ($P \leq 0.02$) with clenbuterol treatment, relative to dioxin treatment alone.

disposing of the liver (a minor component of the total carcass) as the most PCDD/F-contaminated food source, the major repository of clenbuterol residues in edible tissue would be removed as well (not considering lung an edible tissue; calves, Meyer and Rinke, 1991; swine, Smith, 2000; broiler chickens, Malucelli et al., 1994).

Comparison of TEQ calculated for fat, muscle, and liver shows similar shifts in PCDD/F body distributions (Table 9). While 78% of the TEQ was found in the liver of PCDD/F-dosed rats, clenbuterol had no effect on the absolute liver TEQ, but it increased the relative amount remaining in the liver to 83%. Similarly, while Morita et al. (1993) found dietary manipulation with various fiber contents or additives, such as cholestyramine and(or) squalane, could change fecal excretion rates of PCDD/F, total liver burdens were unchanged. In contrast, clenbuterol was able to reduce TEQ in fat and muscle by 32% and 14%, respectively (dioxin vs dioxin + clenbuterol, Table 9). In the absence of exogenous PCDD/F, clenbuterol reduction of TEQ in fat was less effective (8%), and TEQ in liver increased by 14%. On a total-carcass basis, Morita et al. (2001) reduced most PCDD/F congener body burdens with the addition of chlorophyll to the diet of rats. A confounding factor was that chlorophyll was added to the diet 24 h after dietary dioxin dosing, which may have affected PCDD/F absorption as well as excretion. The net TEQ change and muscle burdens were not reported by Morita et al. (2001).

## Implications

Although clenbuterol was effective in reducing body burdens of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/F) in fat and muscle, we do not know whether clenbuterol will be efficacious in animals used for meat (fish, chicken, swine, and cattle). Therefore, future studies should use an appropriate representative species, realizing that not all of these species show a leanness-enhancing response to clenbuterol. Evaluation in large animals has drawbacks when considering the creation of large amounts of biohazardous waste (due to PCDD/F dosing). In monogastrics, the efficacy of clenbuterol might be enhanced by combining it with a dietary chlorophyll. With the realization of the economic and environmental impact of a PCDD/F-contaminated food supply, it behooves the scientific community to investigate further animal remediation technologies.

## Literature Cited


Bernard, A., F. Broeckaert, G. De Poorter, A. De Cock, C. Hermans, C. Saegerman, and G. Houins. 2002. The Belgian PCB/Dioxin incident: nalysis of the food chain contamination and health risk evaluation. Environmental Res. Section A 88:1–18.

Birnbaum, L. S., and L. A. Couture. 1988. Disposition of octachlorodibenzo-*p*-dioxin (OCDD) in male rats. Toxicol. Appl. Pharmacol. 93:22–30.

Birnbaum, L. S., G. M. Decad, and H. B. Matthews. 1980. Disposition and excretion of 2,3,7,8-tetrachlorodibenzofuran in the rat. Toxicol. Appl. Pharmacol. 55:342–352.

Brewster, D. W., and L. S. Birnbaum. 1987. Disposition and excretion of 2,3,4,7,8-pentachlorodibenzofuran in the rat. Toxicol. Appl. Pharmacol. 90:243–252.

Cartana, J., M. Seques, N. J. Rothwell, and M. J. Stock. 1994. Anabolic effects of clenbuterol after long-term treatment and withdrawal in the rat. Metabolism 43:1086–1092.

Carter, W. J., A. Q. Dang, F. H. Faas, and M. E. Lynch. 1991. Effects of clenbuterol on skeletal muscle mass, body composition, and recovery from surgical stress in senescent rats. Metabolism 40:855–860.

Claeys, M. C., D. R. Mulvaney, F. D. McCarthy, M. T. Gore, D. N. Marple, and J. L. Sartin. 1989. Skeletal muscle protein synthesis and growth hormone secretion in young lambs treated with clenbuterol. J. Anim. Sci. 67:2245–2254.

EPA. 1990. Method 1613: Tetra- through octa-chlorinated dioxins and furans by isotope dilution high resolution gas chromatography/high resolution mass spectroscopy. Environmental Protection Agency, Washington, DC.

Feil, V. J., K. L. Davison, G. L. Larsen, and G. F. Fries. 1997. Pentachlorophenol treated wood as a source of dioxin residues in beef. In: Proc. 5th Livest. Environ. Int. Symp., Bloomington, MN. 2:1004–1009.

Hayward, D. G., D. Nortrup, A. Gardner, and M. Clower, Jr. 1999. Elevated TCDD in chicken eggs and farm-raised catfish fed a diet with ball clay from a southern United States mine. Environ. Res. 81:248–256.

Malucelli, A., F. Ellendorff, and H. H. D. Meyer. 1994. Tissue distribution and residues of clenbuterol, salbutamol, and terbutaline in tissues of treated broiler chickens. J. Anim. Sci. 72:1555–1560.

McKinley, M. K., J. J. Diliberto, and L. S. Birnbaum. 1991. 2,3,7,8-Tetrachlorodibenzofuran (TCDF) pretreatment of male fisher rats alters the hepatic metabolism of a subsequent dose. In: Proc. 11th Int. Symp. on Dioxins and Related Compounds. Research Triangle Park, NC. p 144.

McLachlan, M. S., H. Thoma, M. Reissinger, and O. Hutzinger. 1990. PCDD/F in an agricultural food chain, Part 1: PCDD/F mass balance of a lactating cow. Chemosphere 20:1013–1020.

Meyer, H. H. D., and L. M. Rinke. 1991. The pharmacokinetics and residues of clenbuterol in veal calves. J. Anim. Sci. 69:4538–4544.

Morita, K., H. Hirakawa, T. Matsueda, T. Iida, and H. Tokiwa. 1993. Stimulating effect of dietary fiber on fecal excretion of polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-*p*-dioxins (PCDD) in rats. Fukuoka Acta Med. 84:273–281.

Morita, K., T. Matsueda, and T. Iida. 1997. Effect of dietary fiber on fecal excretion of polychlorinated dibenzo-*p*-dioxins in rats. Jpn. J. Toxicol. Environ. Health 43:35–41.

Morita, K., T. Matsueda, T. Iida, and T. Hasegawa. 1999. *Chlorella* accelerates dioxin excretion in rats. J. Nutr. 129:1731–1736.

Morita, K., M. Ogata, and T. Hasegawa. 2001. Chlorophyll derived from *chlorella* inhibits dioxin absorption from the gastrointestinal tract and accelerates dioxin excretion in rats. Environ. Health Perspect. 109:289–294.

NATO/CCMS. 1988. International toxicity equivalency factors (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. Report no. 176. North Atlantic Treaty Organization Committee on the Challenges of Modern Society, Brussels.

Olson, J. R., J. H. McReynolds, S. Kumar, B. P. McGarrigle, and B. P. Gigliottic. 1991. Uptake and metabolism of 2,3,7,8-tetrachlorodibenzofuran (TCDF) in rat hepatocytes and liver slices. In: Proc. 11th Int. Symp. on Dioxins and Related Compounds. Research Triangle Park, NC. p 145.

Perez-Llamas, F., and S. Zamora. 1991. The influence of clenbuterol on growth in rats. Comp. Biochem. Physiol. 99A:241–244.

Pohjanvirta, R., and J. Tuomisto. 1994. Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals: Effects, mechanisms, and animal models. Pharmacol. Rev. 46:483–549.

Reeds, P. J., S. M. Hay, P. M. Dorward, and R. M. Palmer. 1988. The effect of $\beta$-agonists and antagonists on muscle growth and body composition of young rats (*rattus sp.*). Comp. Biochem. Physiol. 89C:337–341.

Schiavetta, A. M., M. F. Miller, D. K. Lunt, S. K. Davis, and S. B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the $\beta$-adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. J. Anim. Sci. 68:3614–3623.

Sillence, M. N., M. L. Matthews, W. G. Spiers, G. G. Pegg, and D. B. Lindsay. 1991. Effects of clenbuterol, ICI118551 and sotalol on the growth of cardiac and skeletal muscle and on $\beta_2$-adrenoceptor density in female rats. Arch. Pharmacol. 344:449–453.

Smith, D. J. 2000. Total radioactive residues and clenbuterol residues in swine after dietary administration of [$^{14}$C]clenbuterol for seven days and preslaughter withdrawal periods of zero, three, or seven days. J. Anim. Sci. 78:2903–2912.

Van den Berg, M., J. De Jongh, H. Poiger, and J. R. Olson. 1994. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. Crit. Rev. Toxicol. 24:1–74.

Van den Berg, M., C. Heeremans, E. Veenhoven, and K. Olie. 1987. Transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to fetal and neonatal rats. Fundam. Appl. Toxicol. 9:635–644.

Wirsling, J. M., K. W. Schramm, A. Kettrup, L. W. D. Weber, and K. Rozman. 1996. Half lives of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin after EROD-inducing and non-inducing doses. Organohalogen Compounds 29:400–405.

WHO. 1997. WHO toxic equivalency factors (TEFs) for dioxin-like compounds for humans and wildlife. World Health Organization, Stockholm, Sweden.